ISSN: 2347-5129
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.352
IJFAS 2016; 4(3): 293-297
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www.fisheriesjournal.com
Received: 29-03-2016
Accepted: 30-04-2016

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Effects of feeding frequency on growth, feed conversion ratio, survival rate and water quality of white leg shrimp (*Litopenaeus vannamei*, Boone, 1931)

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Abstract
This study is conducted to evaluate the effect of feeding frequency on growth, feed conversion ratio and survival rate of white leg shrimp (*Litopenaeus vannamei*) post-larvae in 56 days. Final mean weights obtained for 2 and 4 times/day (6.73 ± 0.09 g and 6.98 ± 0.15 g, respectively) were significantly lower than treatment 6 times/day (8.56 ± 0.16 g) (P<0.05). There was significant difference for feed conversion ratio (FCR) of treatment 6 times/day with treatments 2 and 4 times/day (P<0.05) and the best FCR value was obtained from treatment 6 daily feeding. Special growth rate (SGR) of shrimps were fed 6 times /day was significantly higher than other types of feeding (3.05 ± 0.05%, P<0.05).Best survival rate was obtained in 6 times per day treatment. Salinity, dissolved oxygen and temperature had no difference among treatments (P>0.05). Although ammonium, nitrate, phosphate and pH had significant differences among treatments (P<0.05).

Keywords: *Litopenaeus vannamei*, feeding frequency, FCR, water quality

1. Introduction
White leg shrimp (Litopenaeus vannamei Boone, 1931), native of Central American waters, is the most important aquaculture shrimp species in the world [5, 20]. White leg shrimp was introduced as an aquaculture species in Asia and especially China during mid-year 1990 [21]. At present, white leg shrimp culture involve major contribution of shrimp culture in the world. Therefore, several studies about culture techniques of this shrimp are essential [3]. Feed cost is a limited factor in shrimp culture industry, so attention to selection of a suitable food is essential [2]. Diet has a direct relation with stocking density in white leg shrimp, in fact, if a suitable diet has been considered for this shrimp but ignoring of the stocking density, the desired results cannot be obtained [24]. The daily diet value, feeding time and frequency are key factors in nutrition management strategies that influence on growth and FCR [9, 6, 11]. The highest levels of protease, amylase and lipase in shrimps are at 8, 12 and 20 hours which are the best times of feeding and achieved the highest level of yields per area [25]. Also, automatic feeders which distribute low level of feed in a consistent program can ensure that shrimps are fed on time and in general, 4 to 6 feedings in 3-4 h trials during day to night for juveniles >1 g can be more useful, in addition it must be noted that diet guidelines and management are effective on water quality and shrimp health [4]. The discharge of enriched water of farms into coastal water is one of the most important environmental concerns in shrimp culture which can result in disrupting ecosystem balance [7]. Most of the wastes from drainages of semi-intensive and intensive shrimp culture have high organic loads which originate from formulated feed and unprincipled fertilizing [8]. Therefore, efforts should focus on optimizing and increasing production and decreasing environmental wastes to improve nutrition management [17]. In this study, according to the nutrition importance in white leg shrimp culture, have been investigated the effects of feeding frequency on growth index, feed conversion ratio, survival and water quality of white leg shrimp (Litopenaeus vannamei).

2. Materials and Methods
This study was done in wet laboratory of Zist Pazhohan Company (Khouzestan province, Iran) during 8 weeks from June to August 2015. In this study, it was investigated how feeding

frequency affect growth and water quality. 2, 4 and 6 times feeding frequency treatments during a 24 h period were conducted. 12 indoor circular polyethylene tanks (350 l) with 4 replicate for each treatment were used. Initially, tanks were washed, disinfected and filled up with sea water. Central aerator and air stone were used for better aeration and making a circulation water which resulted in waste accumulating at center. Based on treatments, shrimps (mean weight 1.54 ± 0.05 g) were captured by cast net and stocked 20 individuals in each tank.

Indoor photoperiod was considered 14 h light/ 10 h dark. Shrimps were fed by commercial pellet diets (CP co., Thailand). Diets were given to treatments 2 times/ day (7 and 19 h), 4 times /day (7, 11, 19, 23 h) and 6 times /day (7, 10, 13, 17, 20 and 23 h) during a 24 -h period [22, 25]. In all treatments, 1-10 g shrimps were fed by similar commercial diets. The diet is consisted of 95% dry matter, 42% protein, 5% ash and 9.5% total fat. Diets were given to 1-10 g shrimps based on 4-5% of body weight [1]. Apart from feeding frequency, diet was adjusted in order to estimate feed consumption and prevent from nutrition loss. Residual feed, feces and wastes were daily removed before feeding. All shrimps in each tank were sampled for biometric (weight and length) every two weeks at 6 morning. Also, major water quality parameters (dissolved oxygen, temperature, salinity and pH) were daily recorded. NO_3 and NO_2 were measured by [12] method and ammonium by [18]. Also, 20% water was daily exchanged.

Final survival percent, special growth rate, daily growth index, daily growth rate, percent body weight increasing, final body mass, feed conversion ratio and longitudinal dispersion coefficient were calculated according to following formulas [25]:

Survival percent= (remained shrimps number/ stocked shrimps number) ×100

SGR= (ln final weight – ln initial weight / time) ×100

DGI= (final weight $^{1/3}$- initial weight $^{1/3}$) ×100/day)

DGR= (initial weight × (final weight – initial weight) ×100 / day)

PBWI= (final weight – initial weight) × 100 / initial weight)

FB= final weight × survival,

FCR= food intake/ weight gain and CV= (standard deviation / mean length) × 100, respectively [27, 23]. This experiment was performed in a completely randomized design (Tanks arrangement was RCBD).

2.1. Statistical analysis

Data in each stage were recorded and charts were drawn by Excel (2007). Data analysis was done by SPSS-18 (SPSS® version 18. Chicago, IL, USA) and One-way ANOVA was used to compare growth rate, special growth rate (SGR), daily growth index (DGI), daily growth rate (DGR), percent body weight increasing (PBWI), feed conversion ratio (FCR), survival, final biomass and water quality parameters among treatments and Duncan's multiple range test at 5% significant level was used to compare the mean differences among treatments.

3. Results

During 8 weeks of white leg shrimp rearing (*L.vannamei*), temperature, pH, salinity and dissolved oxygen were measured and recorded 3 times in each 24-h. Water temperature in all treatments was constant without any significant change (29.69 ± 0.09 ºC, n=672, figure.1), also salinity (37 ± 0.71‰ ppt, n=672) and dissolved oxygen (6.62 ± 0.12 ppm, n=672) had no significant changes. In treatment of 6 times/day feeding, pH was significantly lower than treatments 2 and 4 times/day (P<0.05, table 1). Ammonium, nitrite and nitrate levels had significant differences in treatments 2, 4 and 6 times feeding and the lowest was obtained in treatment 6 times feeding (P<0.05, table 1). Phosphate level in treatments 2 and 4 feeding was significantly more than treatment 6 feeding (P<0.05, table 1).

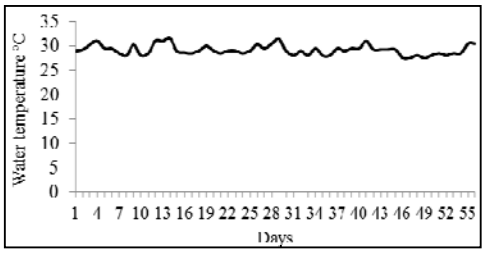

Fig 1: Daily changes of water temperature during rearing period (Mean ± S.E)

Table 1: Water quality parameters (Mean ± S.E)

parameters	Treatments (feeding times/day)					
	2	4	6	F	D. f.	Sig.
pH	8.36±0.09^a	8.28±0.09^a	8.05±0.08^b	25.84	3	*
NH_4^+ (mgl^{-1})	2.15±0.15^a	1.69±0.1^b	1.19±0.3^c	12.16	3	*
NO_3^- (mgl^{-1})	0.29±0.03^a	0.23±0.03^b	0.17±0.02^c	15.50	3	*
NO_2^- (mgl^{-1})	0.23±0.01^a	0.18±0.01^b	0.13±0.02^c	12.67	3	*
PO_4^{-3} (mgl^{-1})	0.03±0.01^a	0.03±0.01^a	0.01±0.0^b	17.50	3	*

*: Significance at 5% level, Different letters show significant differences at 0.05 level (P<0.05)

White leg shrimp (*L. vannamei*) had continuous and stepped growth during the study (table 2). Weight gain of shrimps during growth period showed significant difference among treatments (p<0.05). Shrimps were fed 6 times/day showed best performance. Weight gain in treatment 6 times/day feeding was significantly higher than treatments 2 and 4 times/day (p<0.05). In this study significant difference also was observed in FCR, final biomass, survival, special growth rate, daily growth index, daily growth rate, percent of body weight increasing (p<0.05) (table.3). FCR in shrimps fed 6 times/day compared with 2 and 4 times/day was significantly lower (p<0.05). In treatment 6 times/day, survival and final biomass as well as SGR were significantly more than other treatments (p<0.05). Treatment 6 times/day had the highest value of daily growth index and treatments 2 and 4 had the lowest value (p<0.05). Daily growth rate showed significant increase in treatment 6 times/day compared with treatments 2 and 4 times/day (p<0.05). In addition, percent of body weight increasing in treatment 6 times/day had highest value than the other treatments (p<0.05).

Table 2: Mean growth of shrimp (*L. vannamei*) in every two weeks (Mean ± S.D)

Days	Treatments (feeding times/ day)			
	2	4	6	Sig.
14	3.60±0.05^{c}	3.67±0.05^{b}	4.18±0.06^{a}	*
28	4.62±0.05^{c}	4.68±0.05^{c}	5.78±0.06^{a}	*
42	5.72±0.04^{b}	5.77±0.06^{b}	7.53±0.2^{a}	*
56	6.73±0.09^{c}	6.98±0.16^{c}	8.65±0.17^{a}	*

*Significance at 5% level, different letters show significant differences at 0.05 level (P<0.05)

Table 3: Mean growth index of shrimp (*L. vannamei*) in different feeding treatments at the end of rearing period (Mean ± S.D)

Parameters	Treatments (feeding time/day)					
	2	4	6	F	d.f.	Sig.
Initial weight (gr)	1.54±0.06^{a}	1.53±0.05^{a}	1.56±0.05^{a}	1.33	3	ns
Weight gain (gr)	6.73±0.09^{c}	6.98±0.15^{c}	8.56±0.16^{a}	377.04	3	*
Total length(cm)	10.81±0.08^{b}	11.20±0.1^{b}	12.34±0.09^{a}	310.36	3	*
PBWI (%)	337.01±14.40^{b}	356.20±22.43^{b}	448.71±12.11^{a}	132.05	3	*
SGR (%)	2.65±0.07^{b}	2.70±0.2^{b}	3.05±0.05^{a}	121.6	3	*
DGI (%)	3.08±0.08^{c}	3.23±0.13^{c}	4.17±0.1^{a}	321.07	3	*
DGR (%)	14.27±0.25^{b}	14.89±0.39^{b}	19.50±0.03^{a}	132.05	3	*
FB	125.85±5.26^{c}	130.52±6.8^{b}	169.48±5.98^{a}	110.02	3	*
FCR	2.60±0.06^{a}	2.57±0.05^{a}	1.81±0.04^{b}	147.28	3	*
Survival (%)	85±4.08^{c}	85±8.08^{b}	90±5.08^{a}	20.98	3	*

Ns: no significance, * Significant at 5% level, different letters show significant differences at 0.05 level (P<0.05)

PBWI= percent body weight increasing
DGR= daily growth rate
DGI= daily growth index
SGR= specific growth rate
FB= final biomass
FCR= feed conversion ratio

4. Discussion

The results of this study showed that white leg shrimp (*L.vannamei*) would have faster growth when fed with commercial pellets. It was also determined that shrimps in different treatments had different growth rates. In addition, other factors associated with growth and production showed differences among treatments. There were significant differences in final weight, survival, final biomass, FCR, daily growth rate and percent of body weight increasing of shrimps in all treatments. Moradizadeh *et al* [14] in study on the effects of feeding frequencies on growth rates, FCR and survival rate of Indian white shrimp (*Penaeus indicus*) observed that final weight gain in 6 and 8 times per day treatments were significantly more than 2 and 4 times per day treatments which is in agreement with our findings. Similarly, in a research on the effects of feeding frequency on growth and water quality of white leg shrimp (*L. vannamei*) have been found that shrimps showed better growth when feeding frequency increases from 1 to 4 times per day [26] which agrees with findins of this study. On the other hand, Robertson *et al* [19] indicated that growth rate of *L. vannamei* with initial weight of 6.7 g in earthen ponds increased gradually as feeding frequencies increased from 2 to 4 times per day. While Velasco *et al* [25] by a study on *L. vannamei* post-larvae with less mean weight (initial weight was 0.5 – 0.6 g) in tanks with water exchange system, stated that there was no significant difference in growth rates with different feeding frequencies (1-15 times per day) which is contrary to our findings. Also Smith *et al* [22] reported that there was no difference in the growth of black tiger shrimp (*P. monodon*) when feeding frequencies increased from 3 to 6 times per day. Studies on juvenile shrimps *L. vannamei* have shown that further increase in feeding frequency from 3 and 4 to 7 times per day resulted in final weight reduction [23] which is in contrast to the present study, and it might be due to special laboratory conditions.

The method and protocol of this study is correspond to standard methods by other authors specially Velasco *et al* [25]. It can be stated that less mean weight was reported by Velasco *et al* [25] and Smith *et al* [22] might be related to the period which feed is physically stable in water and also diet ingredients and protein level particularly the combination of free amino acid with appetizing supplements such as betaine (betaine and methionine) which is so effective in feed attraction. It should be noted that if feed stability decreased quickly in water or its attractiveness declined and feed leaching occurred, there is a possible that more frequently fed shrimps would eat more and better than less frequently fed shrimps and this positively affects the growth, since the feeding frequency intervals were more (less meals), feed quality dropped, shrimps used less and consequently had negative effect on growth [10]. Our findings in this study indicate that more feeding frequencies positively affect growth rate. Apart from different feeding treatments in this study, commercial pellets was maintained stable about 3 hours and is available for shrimps. Smith *et al* [22] showed food stability for 12 h. But in general, growth rates of shrimps were desirable even though rearing tanks were small and there was one type of formulated diet. It should be noted that the roles of factors such as salinity, climate and physicochemical conditions of farm water are effective in growth rate.

Results of water quality in this experiment were not consistent with the results of Smith *et al* [22] who showed that water quality did not improve when feeding frequencies increased. While, Moradizadeh *et al* [14] concluded that feed frequency increasing to 6 and 8 times per day had positive effect on chemical factors, which agrees with the results of this study. Similar to Nunes [16] findings on feeding frequency in white leg shrimp, the results of present study showed that in less feeding frequency treatments, more feed amounts were not available to the shrimp because of quality decreasing and caused more water pollution. On the other hand, labor cost for feeding of shrimps might be an important factor in culture

costs [13]. Therefore, increasing of feeding frequency to more than 6 times per day is not cost effective.

This study suggests that increasing the feeding frequency of white leg shrimp (*L. vannamei*) from 4 to 6 times per day results in increasing production and consequently economical profitability. Moreover, decreasing the feeding frequency to less than 6 times per day will have negative effect on water quality and causes increasing of nutrient loads in discharge water of ponds.

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